LINE CHARACTERIZATION AND EVALUATION OF GENETIC PARAMETERS OF SERUM CHOLESTEROL LEVELS, ACTIVITY, FEED INTAKE, GROWTH AND BODY MOISTURE IN SELECTED AND UNSELECTED LINES OF LABORATORY MICE

by

Elizabeth Ann Dunnington

Thesis submitted to the Graduate Faculty of the
Virginia Polytechnic Institute and State University
in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

in

Dairy Science

APPROVED

J. M. White (Chairman)

W. E. Vinson

R. G. Cragle

F. W. Thye

ACKNOWLEDGEMENTS

The author wishes to express her sincere appreciation to Dr. John M. White for his continuous encouragement and guidance throughout the preparation of this thesis. Gratitude is also expressed to the other members of the graduate committee, including Dr. W. E. Vinson, Dr. F. W. Thye and Dr. R. G. Cragle for their suggestions and constructive criticism.

Special thanks are extended to Mrs. Judith Sutphin, Mrs. Jean Dickinson and Mr. Roderick Young for their technical laboratory assistance and to Mrs. Mary Gay Lambert for her aid in the typing of this thesis.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION

Elevated circulating cholesterol levels (CCL) almost certainly contribute to the development of atherosclerosis (Kritchevsky, 1958; Reiser, 1973). The incidence of this vascular disease appears to be increasing in humans. A detailed understanding of factors influencing the levels of circulating cholesterol is essential before effective treatment and preventive measures for the control of atherosclerosis can be determined. Both genetic and environmental factors which influence CCL need to be investigated thoroughly.

Although the ultimate goal of the study of circulating cholesterol levels is to relate results to atherosclerosis in humans, much of the work concerning the modes of inheritance and environmental influences on CCL have involved species other than humans (e.g., mice, rats, chickens, squirrel monkeys). Even though some results may not be directly applicable to the human population, there are several definite advantages to using species other than humans in developing a basic understanding of the genetics, biosynthesis, metabolism and related factors. Some of these advantages are: 1) adaptability to laboratory environments, 2) ease of controlling food intake and activity, 3) relatively inexpensive maintenance costs and 4) comparatively short generation intervals.

The objectives of this study were: 1) to characterize differences in serum cholesterol levels (SCL), voluntary physical activity, feed intake, rate of weight gain and feed efficiency in genetically diverse lines of mice; 2) to estimate the heritabilities of SCL, voluntary physical activity, body weight, weight gain and body moisture content of mice; and 3) to calculate genetic and phenotypic correlations among these traits.

REVIEW OF LITERATURE

Genetic Control of SCL

The genetic aspects of circulating (serum or plasma) cholesterol levels have been studied in several species. Bruell et al. (1962) determined that significant differences occurred in SCL of mice from five inbred strains. He also found a significantly higher SCL in males than in females across all lines. In a later study, Bruell et al. (1963) reported that the mode of inheritance of SCL in mice was intermediate (i.e. neither dominant nor recessive) and additive. This conclusion was reached by performing a diallel cross on five inbred strains of mice and comparing the SCL of the F_1 progeny with the midparent SCL values. The correlation between theoretical (midparent) and observed values was 0.837, indicating that the F_1 progeny values came very close to the midparent values. When the results of reciprocal crosses were pooled, the correlation was even closer.

Similarly, Yamamoto et al. (1963) reported the genetic factors which control plasma lipids concentration (total lipids, cholesterol + cholesterol esters and phospholipids) to be additive in nature and to be influenced by many loci. Three inbred parental strains, their F_1 reciprocal hybrids, backcrosses with the parental lines and reciprocal F_2 crosses from the F_1 lines were employed in the study. Considerable variation in plasma lipid concentration was found among the parental strains. Within litter variances and coefficients of variation were greater in the F_2 crosses than in the F_1 crosses, indicating genetic control. The mean values of total plasma lipid concentration suggested

to the researchers that the genetic variation was additive.

Based on the evidence presented by Yamamoto et al. (1963) and Bruell (1962, 1963), genetic control of circulating cholesterol levels can reasonably be assumed to exist. The next logical step is to determine the extent of genetic control of this trait.

During the development of lines of mice selected for high and low plasma cholesterol levels (PCL) for five generations, Weibust (1973) estimated the realized heritability of plasma cholesterol to be .51 $\pm$ .05 in males and .50 $\pm$ .03 in females. The mice from this study originated from eight unrelated inbred strains which were crossed to produce all possible combinations, resulting in a heterogeneous base population upon which selection for high and low PCL was performed. After only one generation of selection, both male and female means were significantly different in the high, control and low lines.

Eapen et al. (1971) reported a heritability of SCL for male mice to be .73 $\pm$ .47 but did not estimate the value in females due to large sampling errors. Paternal half-sib as well as full-sib family groups were tested for their relative SCL in this experiment.

Although little other information is available on the heritability of cholesterol levels in mice, results have been reported concerning several other species. Estimates of the heritability of SCL in chickens have been reported which ranged from -0.04 to 0.34 (Cherms et al., 1960; Wilcox et al., 1963; and Estep et al., 1969). An estimate of heritability of total serum cholesterol in beef cattle calculated from paternal half-sibs has been reported to be .80 by Stufflebean and Lasley (1969). Clarkson et al. (1971) found squirrel monkeys to have a

realized PCL heritability of .92.

The estimates of heritability of SCL in the literature at present are not in particularly good agreement either among or within species. The degree to which circulating cholesterol levels are influenced by inheritance needs to be estimated more accurately since it may aid in the prevention of atherosclerosis. If inheritance is proved to be a large influencing factor in CCL, people whose close relatives are affected by high levels of circulating cholesterol can be observed more closely and, hopefully, treated more rapidly for the condition, much the same as relatives of diabetics and epileptics are routinely observed more closely for symptoms of those diseases. Thus, one of the major objectives of this study is to estimate as accurately as possible the heritability of SCL.

Relationships Between SCL and Level of Physical Activity

The association between the amount of physical exercise and the concentration of circulating cholesterol has become a point of interest in the general study of cholesterol and atherosclerosis. Before discussing the influence of forced exercise on cholesterol, the mode of inheritance of voluntary activity will be presented.

Employing five inbred lines of mice, Thompson (1956) observed significant differences in levels of exploratory activity among the lines. Two types of apparati, a grid platform and a Y-maze, were used to determine the relative exploratory tendencies of the mice over ten-minute intervals. When activity was graphed as a function of time,

there was only one case of overlap between two of the lines. Except for that one instance, each strain maintained activity scores absolutely apart from the other strains, indicating that hereditary factors definitely influenced the exploratory behavior of mice.

Using a grid apparatus similar to the one mentioned above, McClearn (1961) measured the activity of two inbred strains, their F_1 progeny, backcrosses with the parental strains and F_2 progeny. An increase found in the F_1 mean over the midparent mean was interpreted by the author to indicate the existence of dominance. The data obtained from the F_2 generation led McClearn to estimate that approximately one-half of the F_2 variance in activity was determined by genetic factors.

Bruell (1964) reported the mode of inheritance of spontaneous wheelrunning to be of a heterotic nature. Over a two-year period, 4,000 mice from thirteen inbred strains and thirty-one F_1 populations (randomly chosen) were tested for their willingness to exercise in activity wheels. A significant number of F_1 hybrids in this study obtained activity scores which exceeded the score of their more active parent. Thus, the tendency of these animals to exercise showed "behavior hybrid vigor" which Bruell attributed to either a dominant or over-dominant mode of expression.

Systematic physical training has been cited by many researchers as a method of alleviating high SCL and thus reducing the chances of developing atherosclerosis. The evidence concerning the effects of exercise on circulating cholesterol include opposing conclusions that exercise increases SCL, has no effect on SCL and decreases SCL.

While subjecting rats to a strenuous program of swimming exercise, Papadopoulous et al. (1969) found that plasma cholesterol decreased

gradually. The training regime consisted of a four-hour period of swimming daily for four weeks. Since trained rats consistently had lower PCL than the unexercised control animals, it was concluded that a continuous program of strenuous training resulted in a reduction of plasma cholesterol in rats.

Lewis et al. (1961) reported the effects of exercise on serum lipids in rats which were fed various high fat diets. The form of exercise used was automatically revolving drums in which the rats spent eight hours daily. An interaction was found between exercise and level of fat in the diet. Exercise successfully reduced the level of serum lipids in rats which had suffered elevated serum lipid levels due to a high fat intake.

The relationship between SCL, exercise and food restriction in rats was examined by Jones et al. (1964). Exercise by swimming was found to be effective in preventing an increase in SCL as the animals aged. The authors speculated that exercise might have influenced the correlation between fatness and cholesterol by lowering both.

In another study concerning diet, forced exercise and SCL in rats, Hanson et al. (1967) reported that serum cholesterol was elevated in animals whose calorie intake was restricted as compared to those fed ad libitum and was elevated in exercised animals as compared to sedentary ones. An interaction ($P < .01$) between calorie restriction and exercise occurred. The group of exercised animals for which intake was restricted had the highest SCL, indicating a different relationship between SCL and exercise than the preceding studies.

Similarly, Brainard (1959) reported that rabbits undergoing forced exercise in revolving drums experienced higher serum cholesterol levels than did the sedentary controls. A significant interaction occurred between high levels of cholesterol in the diet and intense levels of exercise.

Obviously the relationship between serum cholesterol and exercise is not a simple one. The evidence presented above gives a reasonable indication that there is an interaction between amount or type of diet and exercise and that other factors are almost certainly involved. For example, laboratory animals which are forced to exercise undoubtedly experience some degree of stress such as being dropped into water and being forced to swim to exhaustion or being coerced into running in a drum by electrical shock or loud noises. Such stress might very well alter the blood chemistry to a much different composition than that of the sedentary controls. The amount of food eaten by exercising animals is often greater than that of sedentary controls. This factor, too, might cause differences in the SCL of the mice.

Relationships Between SCL and Diet

Several studies have been reported on relationships between diet and circulating cholesterol levels. Since altering the dietary intake is a relatively simple treatment, the effects of such a treatment on circulating cholesterol levels are of great interest in the study of atherosclerosis. Due to the magnitude of contradictory literature which exists concerning this subject, a comprehensive report would be lengthy and would serve no useful purpose. Therefore, the objective

here is to report research with laboratory animals which has dealt with the various effects of fasting, calorie restriction, dietary fat and dietary protein on levels of circulating cholesterol.

Kohn (1950) explored the effects of fasting rats for various lengths of time on plasma cholesterol concentrations. Food was withheld from a large group of rats and, each day for a week, a sample group of the animals were bled and discarded. No significant differences in cholesterol levels were precipitated by the fast although there were significant differences among the strains of rats (presumably due to the genetic control of the trait). Sure et al. (1933) also reported that cholesterol levels of rats were not changed by fasting. Conversely, Levin (1945) found that a three-day fast in male rats caused a 28% decrease in cholesterol.

The restriction of food consumption has been examined as it is a more applicable procedure than absolute fasting in altering cholesterol levels. Jones et al. (1964) divided experimental rats into four groups of approximately equal body weight. After obtaining blood samples, one group was sacrificed as a control, one group was exercised and fed ad libitum, a third group was kept sedentary and fed ad libitum, and the last group was kept sedentary and fed a calorie-restricted diet which kept them at an equal mean body weight with the exercised group. It was found that the animals which were fed a restricted diet had total serum cholesterol levels and body fatness almost identical to the animals which were fed ad libitum and kept sedentary. Thus, no lowering of SCL was apparent through calorie restriction in this study.

Hanson et al. (1967) fed one-half of a group of rats ad libitum and the remainder of the animals were fed 65% of the ad libitum consumption for a period of six weeks to ascertain the effects on SCL. Serum cholesterol was significantly higher in those animals receiving a restricted diet than in the ad libitum fed group. Similar findings were reported by Dupont (1965) who concluded that utilization of body stores of fat for energy resulted in accelerated cholesterol biosynthesis.

Although the simple restriction of food consumption has not been found to lower circulating cholesterol levels consistently, evidence has been presented which indicates that CCL fluctuates concurrently with the percentage of fat in the diet. It has not conclusively been proven whether total fat, the saturated portion of the fat, the unsaturated portion of the fat or the ratio of saturated to unsaturated fat actually causes the changes in CCL.

Lewis et al. (1961) fed rats on diets containing unsaturated soya oil or saturated coconut oil. The resulting level of serum lipids was concluded to depend on the saturation of the high fat diets. Those animals fed diets with coconut oil experienced increased serum cholesterol while those fed soya oil maintained normal concentrations of serum cholesterol.

After feeding a high fat diet (29.5% beef tallow, 9.3% corn oil) and a low fat diet (6.9% beef tallow, 2.3% corn oil) to rats for six weeks, Hanson et al. (1967) found a trend toward higher SCL in rats fed the higher fat diet ($P < 0.10$). Since the ratio of animal to vegetable fat in these two diets was almost identical, the results suggested that variation in the total fat of the diet influenced SCL.

A tremendous amount of literature exists concerning the relative effects on circulating cholesterol of saturated versus unsaturated fats, animal versus vegetable fats and the ratios of these types of fat in the diet. In a critical examination of the literature, Reiser (1973) discussed the various misrepresentations and misunderstandings of data concerned with this subject. For example, many researchers have made the incorrect assumption that animal fat and saturated fat are synonymous terms and that animal and/or saturated fat in the diet will inevitably cause serious coronary heart disease and should be largely eliminated from the diet. Although there is persuasive evidence that saturated fat in the diet causes elevated cholesterol even in the absence of dietary cholesterol, this phenomenon (according to Reiser) has not been conclusively proven. He also noted that "... almost all the reports upon which the saturated fat theory is based were made in the late 1950's and early 1960's, during the period when diet cholesterol was thought by many investigators to have no effect."

Another consideration is the tendency for some animals to show an extreme reaction to dietary cholesterol while other animals of the same species have little or no reaction to it. Clarkson et al. (1971) reported such a phenomenon in squirrel monkeys. Two male hyperresponders (animals whose PCL rose drastically after being fed a diet containing cholesterol) and two male hyporesponders (animals which showed no change in PCL due to the same amount of cholesterol in the diet) were each bred with ten to twelve randomly chosen, wild-caught females. The females and twenty-six resulting offspring were then tested for their response to dietary cholesterol. The analysis, which removed biases due to

selection of dams and differences in mean values for parents compared with progeny, indicated clearly that the sires genetically influenced the PCL values of their progeny. Similar genetic control of this excessive response to dietary cholesterol has been demonstrated in humans by Quintao et al. (1971) and in pigeons by Wagner et al. (1973) and Wagner and Clarkson (1974).

An inverse relationship between SCL and the level of dietary protein has been observed in several species. Leveille and Sauberlich (1964) altered the percentage of vitamin-free casein from 9% to 36% for young adult male mice. An increase in the sulfur-containing amino acids or the dietary protein was at least partially responsible for the significantly decreased SCL in all three replicates of this study. The specific mechanisms by which this decrease in SCL occurs is not entirely understood.

Nishida et al. (1960) fed chicks high and low protein diets (35.3% and 17.7% protein, respectively). The results of this study indicated that a low protein diet increased SCL regardless of the type of fat consumed. The evidence was interpreted to mean that the decreased serum cholesterol level of chicks fed the high protein diet was caused by a decrease in cholesterol synthesis and an increased conversion of cholesterol to bile acids.

Coccodrilli et al. (1970) found similar results in male calves which were fed excessive and deficient amounts of protein. While calves on the lower protein diet kept relatively constant blood lipid levels, those consuming the higher protein diet experienced significantly lowered lipid levels. The authors speculated that the dietary protein affected

the cholesterol levels through the liver and gastrointestinal tissue (the two major endogenous sources of cholesterol).

The relationship between diet and circulating cholesterol is quite variable and complex. Reports have indicated that the amount of intake, percentage and saturation of dietary fat and level of dietary protein are all capable of altering the amount of cholesterol which occurs in the circulatory system of an individual.

EXPERIMENTAL PROCEDURES

General Husbandry

The mice in this study were from an ICR albino stock originally from the Institute of Cancer Research, Philadelphia. All of the animals were maintained in a controlled environment laboratory with temperature of 25° C, relative humidity of 50-60% and alternating twelve-hour periods of light and dark. Housing consisted of opaque polypropylene cages approximately 27 cm x 17 cm x 13 cm, with metal cage tops which served as feed hoppers and held water bottles. A commercial bedding and mite dust were used in each cage.

Standard procedures in the rearing of the mice were observed. These procedures included recording the litters at birth, standardizing litters to a fixed number at five days of age (eight pups in the first phase and ten pups in the second phase), permanent identification by toe notching at twelve days of age and weaning at twenty-one days of age.

Phase One - Line Characterization Study

In the first phase of this study a total of 215 mice from two divergent selected lines and an unselected control were used to evaluate differences in SCL, physical activity, growth and feed intake. The lines included a high line (H) which had been selected for 22 generations for maximum rate of 21- to 42-day gain, a low line (L) which had been selected for 19 generations for minimum rate of 21- to 42-day gain, and a control line (C) which was taken from an unselected population of mice

that had been random-bred for 22 generations. Only male pups from litters contributing at least three mice were used. Approximately one-half of the pups from each litter were assigned to an activity study and one-half to a feed intake study.

The activity study measured voluntary physical activity by allowing each mouse free access to an exercise wheel every sixth day of the study from 21 to 70 days of age. Thus, each individual was given nine opportunities to run in the wheel, the first of which was designated an acclimation period and was not used in the analysis. Each cage consisted of a small sedentary space (5 cm x 5 cm x 9 cm) where feed and water were available, and an adjoining wheel approximately 15 cm in diameter and 6 cm wide which revolved freely. The number of revolutions turned by each individual during an activity period of 22 hours was recorded by a mechanical counter and served as the activity score for that period.

The feed intake study measured consumption and rate of gain of the mice from genetically divergent lines. Mice from each litter were randomly assigned to either a high fat diet (11% fat) or a control diet (4-1/2% fat). Although the high fat diet supplied over twice the fat percentage of the control diet, neither diet contained abnormally large percentages of any ingredient or lacked any required nutrient (Table 1). Feed intake and growth rate were obtained by observing the amount of feed consumed over a period of time and the corresponding change in body weight over the same time period. Mice were placed in individual, clean cages at the beginning of each test period to insure that no residual feed was present in the cage and were fed a pellet form of chow which virtually eliminated any loss of feed through sifting. The mice were weighed once

TABLE 1. PERCENTAGE COMPOSITION OF DIETS

Nutrient	Phase One		Phase Two
	Control Diet[1]	High Fat Diet[2]	Intermediate Diet[3]
Protein	23.4	17.0	20.0
Fat	4.5	11.0	9.0
Animal Sources	1.0	3.4	4.5
Plant Sources	3.5	7.6	4.5
Fiber	5.2	2.2	2.5
NFE	50.8	51.7	54.8
Ash	7.3	5.5	4.8
Moisture	10.0	10.0	10.0

[1]Purina Lab Chow [2]Purina Mouse Chow [3]Purina Mouse Chow (Comm.)

[1,2,3]Adequate vitamin supplements are included.

each week and the feed for each animal was weighed in and out three times per week, supplying data for calculation of feed consumption, rate of gain and feed efficiency (= gain/intake) on a weekly basis.

Blood samples for SCL determination were taken from the mice in the activity and feed intake studies at 35 and 70 days of age. Serum cholesterol concentrations at these ages supplied information concerning the change in SCL through the period of maturity and the correlation between samples taken from the same individual as well as differences due to lines and diets. The serum cholesterol level of the mice was determined from approximately 0.5 ml of blood, removed by sinus orbital puncture (Riley, 1960) after a six-hour fast. The fast was imposed to standardize the amount of intake immediately prior to bleeding without an extended period of starvation. The total serum cholesterol concentration was obtained by gas chromatography (Driscoll et al., 1971). In this method of cholesterol determination, the blood sample was centrifuged for thirty minutes. The cleared serum was then siphoned off and mixed with one ml of ethanol and one ml of alcoholic KOH (6 ml of 33% KOH were diluted to 100 ml with ethyl alcohol) on a Vortex mixer. Saponification was allowed to occur for three hours in a 37° C water bath. One-half ml of heptane was added to the sample and mixed, then one ml of water was added and mixed. The heptane fraction was immediately drawn off and injected into an F & M model 400 gas chromatograph. The sample cholesterol peaks were compared with peaks from a pure cholesterol standard which was injected after every second sample. Duplicates were analyzed on any sample which contained a sufficient amount of blood.

The data from this study were analyzed by least squares. For the activity study, the model included lines as the main effect. For the feed intake study the model included lines, diets and line x diet interactions.

Phase Two - Evaluation of Genetic Parameters

In this portion of the study, the random bred control line was used to determine heritabilities of and genetic correlations among body weight, weight gain, SCL, activity and body moisture content. A generation of males was raised, tested and used to sire a generation of offspring which were then tested. This experimental design allowed the use of a sire-son regression procedure for estimating heritability.

In the sire population, 335 males were tested, then bred randomly to a population of females in the ratio of one male to three females at approximately 80 days of age. In order to be used in the analysis, each male had to sire at least two litters from which groups of at least two full brothers survived. The son generation was then raised and tested exactly as the sire generation had been tested.

Activity was measured in exercise wheels for a 22-hour period when each mouse was 28, 49, and 70 days of age. Since the number of activity cages was the limiting factor in determining how many animals could be tested and the correlations between activity scores of each individual were high in the first phase of the study (.37), it was decided that three activity periods would supply adequate data concerning willingness to exercise while allowing a sufficiently large number of animals to be tested.

Blood samples were drawn from the animals and analyzed for SCL by the procedures described previously. The high correlation (.40) between SCL at five and at ten weeks of age in the first phase of the study indicated that one sample per individual would provide sufficient data in this study. This sample was taken at 62 days of age since, at that age, the mice were sexually mature and were not being tested for activity.

After all testing was complete, the mice in the genetic phase of the study were maintained until they reached 116 to 125 days of age. At this time they were fasted for 16 hours, weighed and overdosed with ether. The carcasses were immediately wrapped in individual plastic bags and frozen (4° C) for at least 24 hours. The frozen carcasses were then chopped into one to two cm wide slices and lyophilized in a Virtis freeze dryer. Using the empty live weight as a basis, the moisture content of each individual was calculated.

A sizable inverse relationship between the percentage of body fat and body moisture has been reported in laboratory mice. Lang and Legates (1969) estimated this correlation to be -.95, and White (1974) reported a value of -.71. Therefore, a reasonable estimate of the comparative body fat percentages can be obtained by observing the percentage of the body which is composed of water. Since the number of animals used in this phase of the study was large (660 mice) and total body composition is a lengthy, tedious procedure, the method of lyophilizing carcasses to obtain body moisture content was chosen and conservative inferences about relative fat percentages were drawn.

Methods of Analysis

Heritability is, according to Falconer (1960), the proportion of the total phenotypic variance of a trait that is attributable to the additive genetic variance. Thus, $h^2 = \frac{V_A}{V_P}$, where h^2 = heritability, V_A = the additive genetic variance and V_P = the total phenotypic variance. This parameter can also be defined as the regression of breeding value on phenotypic value, $h^2 = b_{AP}$.

Several methods of estimating the heritability of traits have been employed, including the regression of offspring on one parent, regression of offspring on mid-parent, half-sib correlations and full-sib correlations. Beyond the practical aspects of the types of relatives available for study, sampling errors and environmental sources of covariance affect the statistical precision of the estimate. In very general terms, the half-sib correlation and regression of offspring on sire appear to be more reliable than the other procedures. Maternal effects may bias the estimate of heritability based on an offspring-dam regression in litter-bearing animals and the influence of environmental correlations and maternal effects render the full-sib analysis the least accurate of these four methods. Because of the difficulties involved in producing a sufficient number of half-sibs in a laboratory mouse population to accurately estimate genetic parameters, the sire-son analysis technique was chosen to estimate heritability in this study. The regression of offspring on one parent is a measure of $1/2h^2$, so that $h^2 = 2b_{YX}$, where b_{YX} = the regression of son on sire.

Kempthorne and Tandon (1953) treated in detail the estimation of heritability by regression of offspring on parent. They cited three methods of estimating heritability when the number of offspring per parent is not a constant (as is normally the case in practical application).

The first procedure is the regression of offspring on parent, in which the parent's record is repeated for each of his offspring. The second method is the regression of the average of offspring records on the parent's record. Procedure one would be accurate if the correlation among the offspring of a parent were zero, while procedure two assumes each progeny group correlation to be one. In most practical situations, repetition of the parent's record is more accurate than averaging the offspring records because the progeny group correlation is usually closer to zero than to one.

A third method to consider is an intermediate system whereby the progeny means are weighted according to the number of progeny and an estimated value of ρ (where ρ = a correlation coefficient between deviations from regression associated with two progeny of the same parent). In data involving dairy cattle, Kempthorne and Tandon (1953) found little difference in the variances of the three estimates.

Bohren et al. (1961) evaluated the relative efficiencies of the three heritability estimates mentioned above. After investigating the nature and magnitude of the correlation coefficient, and comparing the relative efficiencies of the various techniques in data from a population of poultry, they concluded that the regression of the average of

n offspring values on the parent value was subject to the largest statistical errors. The other two methods were of virtually equal efficiency, indicating that there was little advantage in using the weighted technique rather than repetition of the parent's record. Therefore, the estimates of heritability found in this study were calculated by a sire-son regression in which the records of the sires were repeated for each of their sons.

Phenotypic correlations (r_p) were calculated using the general formula from Hazel (1943):

$$r_p = \frac{\text{cov } (P_1, P_2)}{\sqrt{\sigma^2_{P_1} \quad \sigma^2_{P_2}}}$$

where cov (P_1, P_2) = the phenotypic covariance between trait 1 and trait 2

$\sigma^2_{P_1}$ = the variance of trait 1 (pooled over sires and sons)

$\sigma^2_{P_2}$ = the variance of trait 2 (pooled over sires and sons)

Genetic correlations (r_g) were calculated by the arithmetic method of Becker (1967) according to the following formula:

$$r_g = \frac{\text{cov}_{X_1 Z_2} + \text{cov}_{X_2 Z_1}}{2\sqrt{\text{cov}_{X_1 Z_1} \quad \text{cov}_{X_2 Z_2}}}$$

where $cov_{X_1Z_2}$ = the covariance of trait 1 in the sires with trait 2 in the sons.

$cov_{X_2Z_1}$ = the covariance of trait 2 in the sires with trait 1 in the sons.

$cov_{X_1Z_1}$ = the covariance of trait 1 in the sires with trait 1 in the sons.

$cov_{X_2Z_2}$ = the covariance of trait 2 in the sires with trait 2 in the sons.

The formula used to estimate the standard error of the genetic correlation was from Reeve (1955), Robertson (1959), and Becker (1967).

$$S.E._{(r_g)} \simeq \frac{1 - r_g^2}{\sqrt{2}} \sqrt{\frac{S.E.\ (h_1^2)\ S.E.\ (h_2^2)}{h_1^2\ h_2^2}}$$

where S.E. (r_g) = the standard error of the genetic correlation.

r_g = the genetic correlation.

S.E. (h_1^2) = the standard error of the heritability of trait 1.

S.E. (h_2^2) = the standard error of the heritability of trait 2.

h_1^2 = the heritability of trait 1.

h_2^2 = the heritability of trait 2.

RESULTS AND DISCUSSION

Phase One - Line Characterization

A characterization of differences in serum cholesterol levels, voluntary physical activity, feed intake, rate of gain and feed efficiency was performed with genetically diverse lines of mice. The 215 male mice originated from three lines, two of which had been selected for high and low rate of weight gain from 21 to 42 days of age. The third line was an unselected control population.

Voluntary Physical Activity

The willingness to exercise voluntarily was measured in 113 mice from the three lines through the use of activity wheels. The activity scores were found to differ significantly among lines (Table 3). Those mice selected for low rate of gain consistently exercised on the activity wheel much more than the other animals in the study. The mean activity score of the control line was, for each period, intermediate and the high line mice were the most lethargic (Table 2 and Figure 1). These significant line differences agree with previous literature in indicating that the willingness to exercise is genetically controlled (Thompson, 1956; McClearn, 1961; Bruell, 1964).

Many studies involving humans, rats and other species have reported that animals of lean body form are normally more active and are more successful in undergoing a strenuous activity regime than heavier individuals (Falconer, 1953; Kritchevsky, 1958). The low, control and high line mice gained an average of 12.3, 20.4 and 35.5 grams, respectively, during the course of the study (from 21 to 70 days of age). Thus, the average

TABLE 2. MEANS, STANDARD DEVIATIONS AND COEFFICIENTS OF VARIATION OF ACTIVITY SCORES -- LINE CHARACTERIZATION STUDY

Age (Days)	High Line (40)[1]			Control Line (36)			Low Line (37)		
	Mean	S.D.	C.V. (%)	Mean	S.D.	C.V. (%)	Mean	S.D.	C.V. (%)
27	3,018[2]	1,694	56.1	5,818	3.151	54.2	6,635	4,838	72.9
33	6,767	2,350	34.7	11,188	4,909	43.9	13,123	5,397	41.1
39	10,311	4,634	44.9	15,248	5,909	38.8	18,827	5,678	30.2
45	10,037	6,966	69.4	14,279	6,529	45.7	15,719	7,218	45.9
52	11,889	5,495	46.2	14,818	6,853	46.2	17,231	7,956	46.2
58	13,408	8,756	65.3	17,751	8,894	50.1	19,093	9,502	49.8
64	11,991	6,611	55.1	15,974	6,334	39.7	17,983	7,024	39.1
70	11,813	6,056	51.3	13,078	5,983	45.8	15,797	6,438	40.8
TOTAL	79,234	28,374	35.8	107,987	28,177	26.1	124,409	32,896	26.4

[1](N) - number of observations per mean.

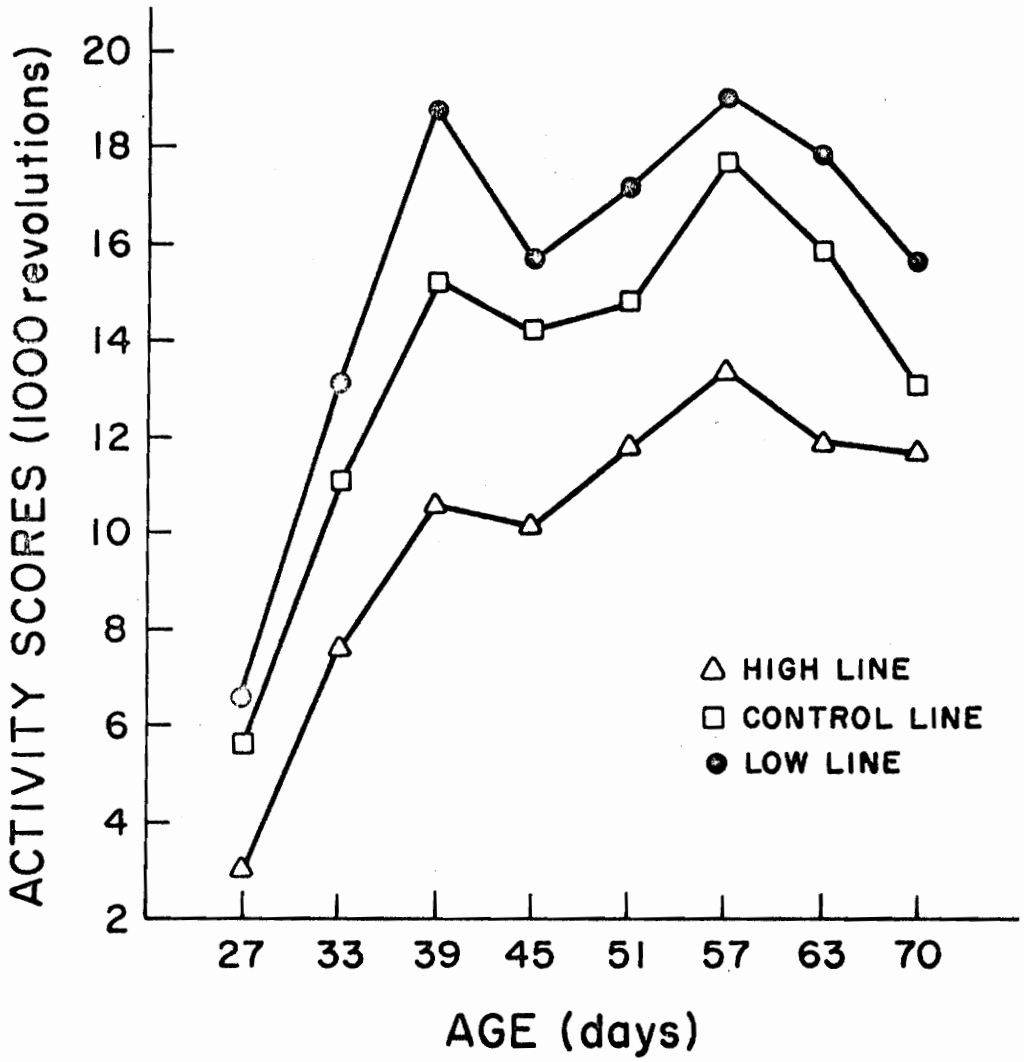
[2]revolutions of exercise wheel per activity period.

TABLE 3. ANALYSIS OF VARIANCE OF ACTIVITY -- LINE CHARACTERIZATION STUDY

Source	df	MS	F
Lines	2	20,221,172,569	22.7**
Error	110	892,213,875	

**(P < .01).

FIGURE 1. LINE DIFFERENCES IN VOLUNTARY PHYSICAL ACTIVITY



size of the animals in each line was quite distinct and the lighter mice did exercise considerably more than the heavier mice.

Peaks of activity occurred in all three lines at 39 days of age and again at 57 days of age. The peak at 39 days of age coincided with the period of maximum feed consumption of the mice in the feed intake study and was the approximate time of sexual maturity for these animals. The low and control lines showed more variable activity scores than the high line, increasing more rapidly during the first two weeks of the test and dropping off more sharply during the last two weeks.

Feed Intake, Growth and Efficiency

A feed intake study was performed with 102 mice from the selected and unselected lines. From weaning until the end of the test, one-half of the mice in each line were fed a high fat diet (11% fat) and the remaining mice were fed a control diet (4-1/2% fat). (See Table 1 for an approximate analysis of these diets.) The feed intake study resulted in highly significant differences both among lines and between diets and a significant line x diet interaction in feed efficiency occurred (Tables 4 and 5). The high line consumed the greatest amount of feed during the course of the test, the low line ate the least and the control line consumed an intermediate amount (Figure 2). The high line gained considerably more weight than the controls and the low line gained considerably less during the seven-week test (Figure 3). The results indicated that the selection for high and low weight gain in developing the lines of mice had been effective. On a weekly basis, however, the low line began gaining more weight than the control line after about 50 days of age. This suggests that selection for low

TABLE 4. MEANS AND STANDARD ERRORS OF INTAKE, GAIN AND EFFICIENCY -- LINE CHARACTERIZATION STUDY

Line	Diet	(N)[1]	Total Feed Intake (g)	Total Weight Gain (g)	Total Feed Efficiency %
H	Control	(14)	363.2 ± 8.0	33.7 ± 1.2	9.3 ± 0.3
	High Fat	(16)	316.5 ± 6.1	37.0 ± 0.3	11.7 ± 0.3
C	Control	(20)	293.2 ± 4.9	19.9 ± 0.6	6.8 ± 0.2
	High Fat	(20)	264.8 ± 8.2	20.8 ± 0.7	7.9 ± 0.3
L	Control	(15)	238.0 ± 8.1	11.6 ± 0.3	4.9 ± 0.2
	High Fat	(17)	212.0 ± 4.4	12.9 ± 0.7	6.1 ± 0.2

[1](N) = number of observations per mean.

TABLE 5. ANALYSIS OF VARIANCE - FEED INTAKE, GAIN AND EFFICIENCY -- LINE CHARACTERIZATION STUDY

Source	df	Feed Intake		Rate of Gain		Feed Efficiency	
		MS	F	MS	F	MS	F
Lines	2	101,699.2	130.9**	4,235.6	440.5**	.0201	189.7**
Diet	1	28,463.5	36.6**	84.2	8.8**	.0064	60.5**
Line x Diet	2	1,000.6	1.3	13.1	1.4	.0004	4.1*
Residual	96	777.1		9.6		.0001	
TOTAL	101						

*(P < .05) **(P < .01)

FIGURE 2. TOTAL FEED CONSUMPTION -- LINE CHARACTERIZATION STUDY

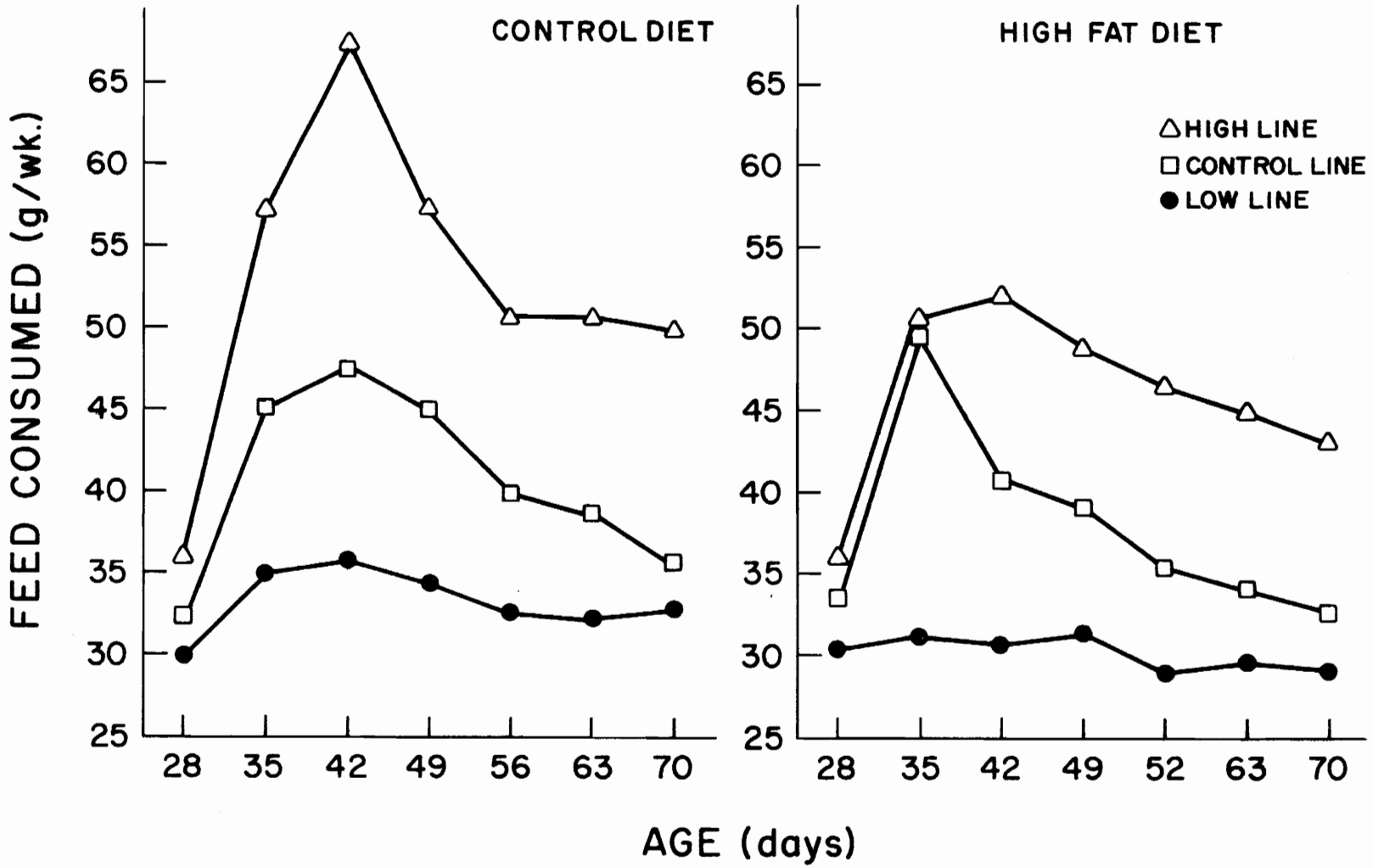
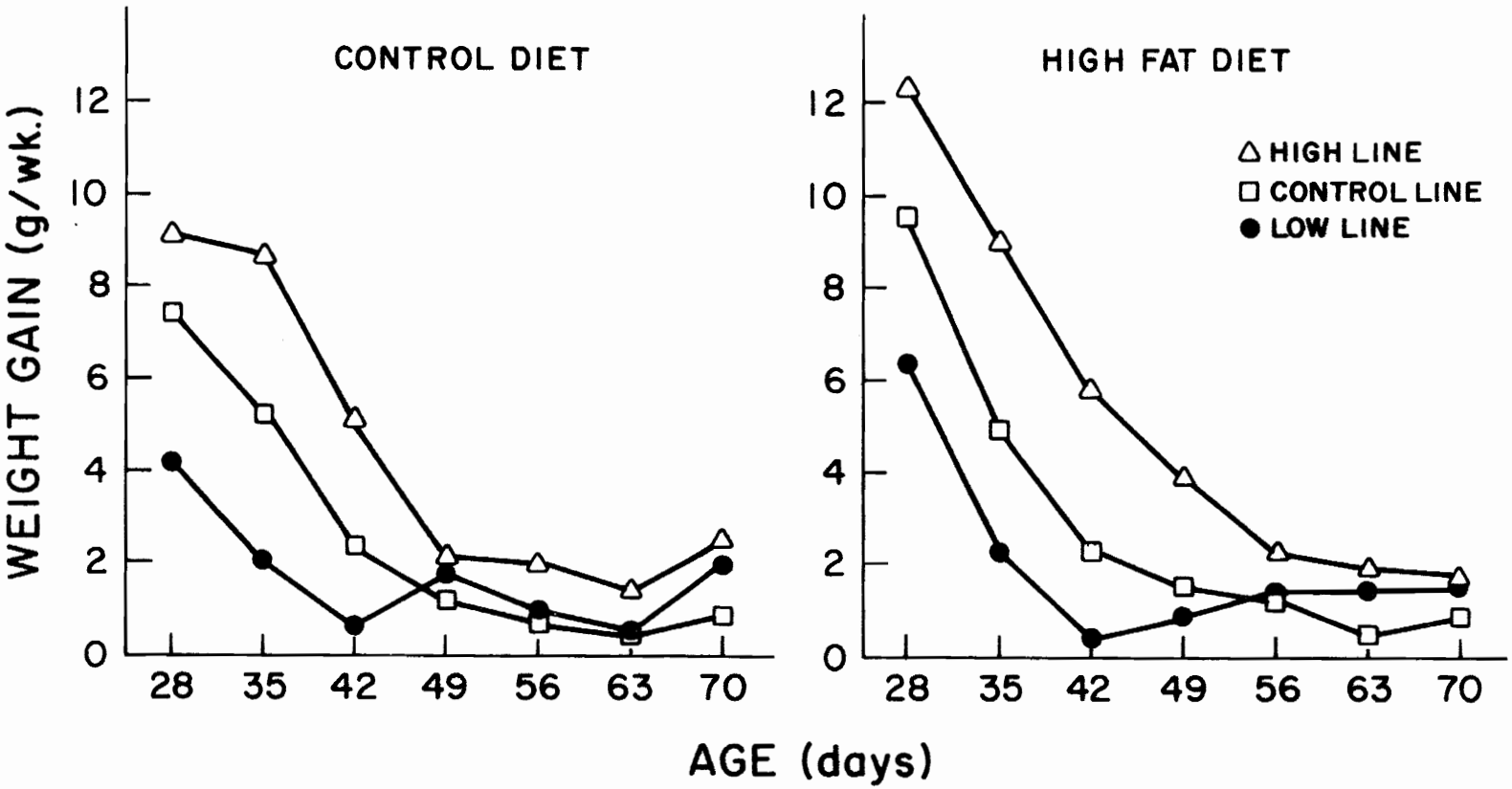


FIGURE 3. TOTAL GAIN IN BODY WEIGHT -- LINE CHARACTERIZATION STUDY

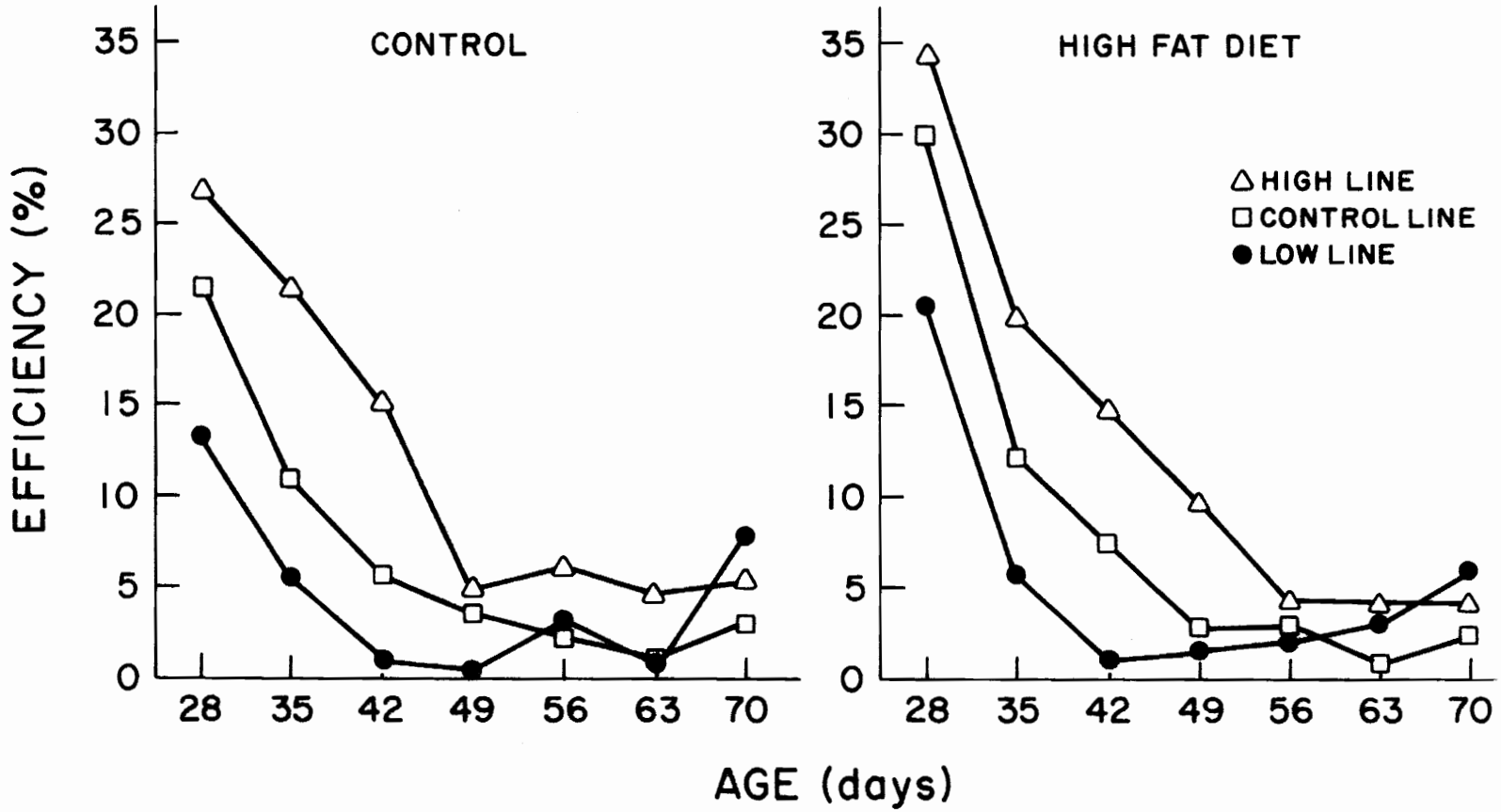


weight gain from 21 to 42 days of age might favor those animals which mature more slowly (i.e., sometime after 42 days of age).

The total efficiency of the high line was very nearly double that of the low line and the control line maintained an intermediate level (Figure 4). A significant interaction ($P < .05$) occurred between line and diet in total efficiency. Since the high line mice were relatively lethargic, it is logical to assume that they expended less energy than low line mice in supporting their level of activity and, thus, gained weight with greater total efficiency. Generally, the low line mice consumed relatively smaller amounts of feed and exercised more, resulting in a lower efficiency. However, the low line mice began to gain weight more rapidly after 50 days of age but were not consuming any more feed than they had previously, so the efficiency of the low line increased to a level above the high and control lines at ten weeks of age. The consequences of this trend were not evaluated since the mice were not maintained after ten weeks of age. Data collected on these selected lines through old age (i.e., until natural death) would provide interesting insights into the far-reaching effects of such a selection program.

The low line mice were observed to be extremely nervous and hyperactive from weaning until approximately seven weeks of age, after which their level of activity decreased considerably. This observation is used to interpret some of the data. For example, the activity scores of the low line mice dropped more sharply than that of the high line after about 57 days of age, presumably because their nervousness was abating. In the feed intake study, the relative increase in growth and

FIGURE 4. TOTAL FEED EFFICIENCY -- LINE CHARACTERIZATION STUDY



efficiency in the low line after 50 days of age (which was not accompanied by an increase in feed consumption) could have been due to the steadying of the disposition of these mice after about seven weeks of age. That is, they were becoming more calm and, consequently, less active so that consuming the same amount of feed resulted in greater gains and an increased efficiency.

Differences in feed intake and growth measurements due to diet were highly significant (Table 5). In each line a greater amount of the control diet was consumed than the high fat diet (Table 4). This seems reasonable since appetite is thought to be governed by the amount of energy consumed (Maynard and Loosli, 1969). Apparently, a smaller amount of the high fat diet was necessary to fulfill the need for energy and, thus, to satiate the appetite.

More weight was gained per unit time by the mice fed the high fat diet. This result is also reasonable, since one unit of dietary fat supplied approximately twice the caloric content of one unit of protein (Maynard and Loosli, 1969). Since mice fed the 11% fat diet gained more weight while consuming a smaller amount of feed, their efficiency was consistently higher than the animals fed the control diet.

The total digestible nutrients available in the high fat diet and control diet were 88% and 75%, respectively. The means of total feed intake for the three lines and two diets from Table 4 were converted, using the factors above, to total digestible nutrients consumed. Then, total feed efficiencies were calculated from these intake data (Table 6). Although total efficiencies were somewhat higher in general, the high line remained the most efficient, the low line the least

TABLE 6. MEANS OF INTAKE AND EFFICIENCY BASED ON TOTAL DIGESTIBLE NUTRIENTS CONSUMED -- LINE CHARACTERIZATION STUDY

Line	Diet	(N)[1]	Total Digestible Nutrients Consumed (g)	Total Feed Efficiency %
H	Control	(14)	272.4	12.4
	High Fat	(16)	278.5	13.3
C	Control	(20)	219.9	9.1
	High Fat	(20)	233.0	8.9
L	Control	(15)	178.5	6.5
	High Fat	(17)	186.6	6.9

[1](N) = number of observations per mean.

efficient and the control line intermediate.

Serum Cholesterol Levels

The mean values of SCL for all mice in the activity and feed intake studies are shown in Table 7. In every case the SCL at ten weeks of age was higher than the corresponding value at five weeks. This gradual increase in SCL during maturity is a rather well-documented phenomenon (Kritchevsky, 1958).

Highly significant differences among lines occurred in SCL of the mice used in the activity study (Table 8). The SCL of the high line mice were consistently greater than that of the control mice. The low line mice had the highest SCL at five weeks of age but, by ten weeks of age, the low line mean was less than either the high or control lines. From the slope of the lines in Figure 5-A, it is apparent that the cholesterol level of the mice selected for low weight gain increased at a much slower rate than SCL of the high and control lines. The slopes of the lines in Figure 5-A indicate that an interaction between age and line may be present for SCL of exercised mice. Such an interaction may have occurred in the low line because the mean activity scores of this line were much greater than those in the high and control lines (i.e., that particular line of mice exercised so extensively that the physiological balance was changed enough to alter serum cholesterol levels).

Significant differences in SCL at five weeks were also found among lines in the feed intake study (Tables 7 and 8). At five weeks of age the high line had the greatest concentration of SCL, the low

TABLE 7. MEANS AND STANDARD ERRORS OF SCL -- LINE CHARACTERIZATION STUDY

Line	Age (Days)	Activity Study (N)[1]	Activity Study Control Diet	Feed Intake Study (N)	Feed Intake Study Control Diet	Feed Intake Study (N)	Feed Intake Study High Fat Diet
H	35	40	102.2[2] ± 5.5	14	122.1 ± 13.2	16	143.8 ± 11.0
C	35	36	82.1 ± 4.3	20	91.5 ± 6.4	20	123.0 ± 12.2
L	35	37	103.7 ± 4.7	15	97.3 ± 8.3	17	139.5 ± 8.0
H	70	40	155.0 ± 7.8	14	143.3 ± 12.5	16	220.3 ± 20.1
C	70	36	130.5 ± 7.9	20	124.1 ± 11.5	20	178.1 ± 13.3
L	70	37	117.6 ± 5.4	15	132.2 ± 18.0	17	180.4 ± 11.4

[1](N) = number of observations per mean.

[2]mg/100 ml.

TABLE 8. ANALYSIS OF VARIANCE - SCL -- LINE CHARACTERIZATION STUDY

Source of Variation	df	Five Weeks of Age MS	Five Weeks of Age F	Ten Weeks of Age MS	Ten Weeks of Age F
		Activity			
Line	2	5323.7	5.9**	13992.4	7.2**
Error	110	905.0		1935.1	
		Feed Intake, Gain and Efficiency			
Line	2	5643.4	3.3*	8745.9	2.4
Diet	1	25335.4	14.8**	89388.5	24.7**
Line x Diet	2	812.1	0.5	1791.5	0.5
Error	96	1717.0		3615.9	

*(P < .05) **(P < .01)

FIGURE 5. DIFFERENCES IN SCL DUE TO LINE AND ACTIVITY -- LINE CHARACTERIZATION STUDY

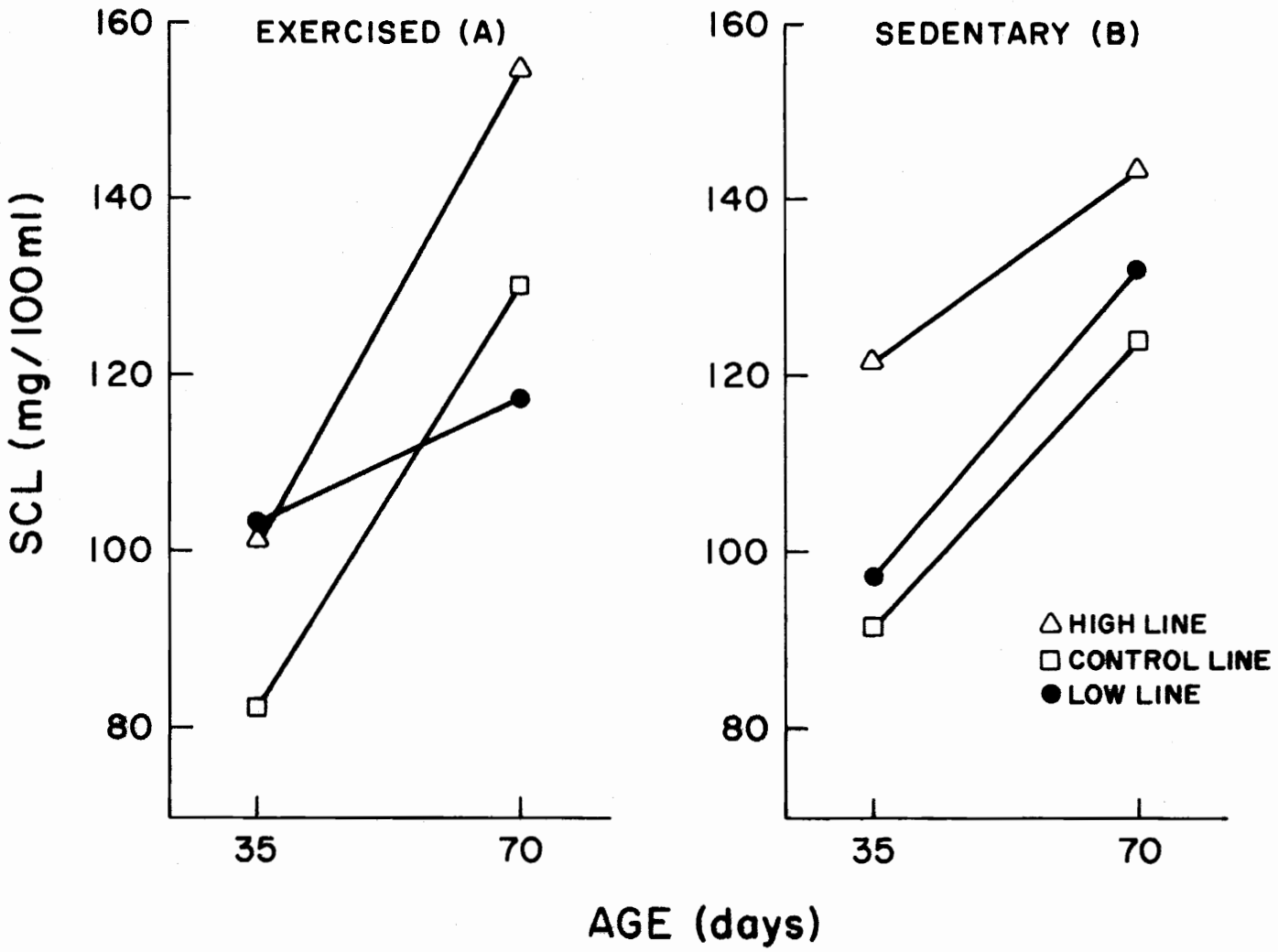
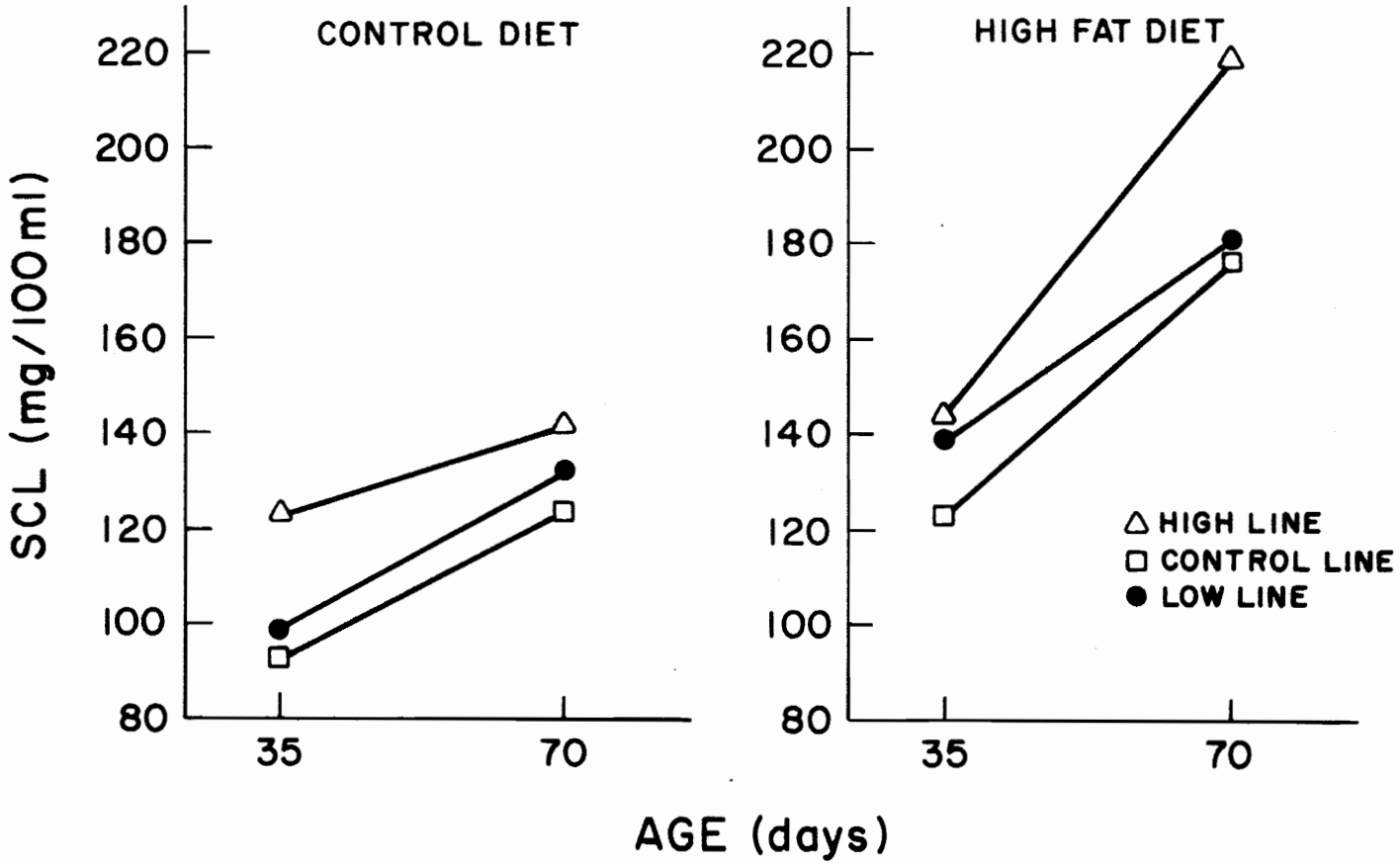


FIGURE 6. DIFFERENCES IN SCL DUE TO LINE AND DIET -- LINE CHARACTERIZATION STUDY



line an intermediate amount and the control line the lowest level (Figure 6). Although the line effect was not significant at 10 weeks of age, the same relationship among SCL in the three lines occurred. In general, animals with lower body mass have lower SCL (Kritchevsky, 1958). In this study the elevated SCL of the low line mice may have been due to the relatively nervous disposition of this line. As mentioned earlier, the nervousness and hyperactivity in the low line occurred from the time of weaning until about seven weeks of age. This condition could explain several of the results obtained in the SCL data. For example, in the activity study the low line mice had SCL above the high and control line SCL means at five weeks of age. By ten weeks of age, however, the low line SCL mean was well below that of the high and control lines. One interpretation of this is that the extremely high exercise level of the low line mice served to reduce their normal increase in SCL with age. Conversely, in the sedentary low line mice of the feed intake study, the elevated SCL relative to the control line, which occurred at five weeks of age, remained high at ten weeks of age, perhaps because those mice were not given the opportunity to exercise on activity wheels. Obviously, the physiological, biochemical, and genetic interactions among the lines, exercise and SCL are complex ones and this study was not designed to differentiate among the various concepts. The pheonomena are, however, very important in the understanding of basic cholesterol metabolism and certainly warrant further research.

The differences in SCL between activity mice (all of whom were fed control diet) and sedentary mice which received the control diet were most distinct in the low line (Figure 5). Since the activity mice

were allowed to exercise about once each week but were not forced into physical training, no general effect due to exercise was expected. The low line mice, however, seemed willing to exercise sufficiently to successfully reduce the normal increase in SCL, as was discussed above. Conversely, the high and control line mice apparently did not exercise sufficiently to retard the increase in SCL from five to ten weeks of age.

The differences in SCL due to diet were quite pronounced (Figure 6). Those animals fed a high fat diet had significantly elevated cholesterol levels compared to the mice consuming the control diet. Unfortunately, the high fat diet was confounded with a lower protein level and a higher fiber content than the control diet, thus making it impossible to determine whether the increased fat consumption, decreased protein consumption, increased fiber consumption or some combination of these factors was responsible for the elevated serum cholesterol levels.

Phenotypic Correlations

Within line phenotypic correlations between the traits measured in this study were calculated. In the activity study the score from each of the eight activity periods was found to be highly correlated with the total activity score and SCL at five and ten weeks of age were positively correlated (Table 9).

SCL and total activity were significantly, negatively correlated at five and ten weeks of age in the high line and at five weeks of age in the low line. The other values between these two traits were not appreciably different from zero.

TABLE 9. PHENOTYPIC CORRELATIONS IN ACTIVITY -- LINE CHARACTERIZATION STUDY

		Activity Period 1	Total Activity	SCL 5 Weeks	SCL 10 Weeks
Activity Period 1	H		.31**	-.11	-.07
	C	1.0	.53**	.02	-.03
	L		.52**	.01	.15
Total Activity	H			-.22*	-.41**
	C		1.0	.13	.07
	L			-.28**	.06
SCL 5 Weeks	H				.42**
	C			1.0	.31**
	L				.23*
SCL 10 Weeks	H				
	C				1.0
	L				

*(P < .05) **(P < .01)

In the feed intake study total correlations (i.e., correlations of all animals with the effects of line removed) were initially calculated. For these total correlations the relationships of SCL with intake, gain and efficiency were not significantly different from zero. Since there were highly significant differences among the lines in all these responses, however, the data were reanalyzed for each line individually (with the effects of diet removed). In this second analysis, differences in the correlations in the three lines became apparent (Table 10).

Total gain was highly, positively correlated both with total intake and with total efficiency in all lines. The correlations between total intake and total efficiency were significant and negative in the high and control lines, agreeing with results reported by Jara-Almonte and White (1973), but significant and positive in the low line.

SCL at five weeks of age was highly correlated with SCL at 10 weeks of age, as was found in the activity study. Interesting differences in correlations due to lines were found to exist between SCL and the feed intake traits. At both five and ten weeks of age in the high line, highly significant, positive correlations were found between efficiency and SCL while a highly significant, negative correlation occurred between intake and SCL. This seems to indicate that the animals which experienced very high levels of SCL were, in general, those which were efficient in gaining weight rather than those which consumed large amounts of feed inefficiently. Conversely, the mice in the low line demonstrated a different trend in correlations. The only two correlations which were significant in the low line occurred between gain and SCL at five weeks

TABLE 10. PHENOTYPIC CORRELATIONS IN FEED INTAKE -- LINE CHARACTERIZATION STUDY

		Total Intake	Total Gain	Total Efficiency	SCL 5 Weeks	SCL 10 Weeks
Total Intake	H		.43**	-.33**	-.37**	-.41**
	C	1.0	.30**	-.37**	-.10	.01
	L		.65**	.21*	-.09	-.13
Total Gain	H			.70**	.11	.03
	C		1.0	.76**	.06	.18
	L			.88**	-.22*	-.09
Total Efficiency	H				.39**	.35**
	C			1.0	.11	.21*
	L				-.23*	-.06
SCL 5 Weeks	H					.34**
	C				1.0	.40**
	L					.50**
SCL 10 Weeks	H					
	C					1.0
	L					

*(P < .05) **(P < .01)

and between efficiency and SCL at five weeks. Both of these correlations were negative and significant ($P < .05$). Thus, the mechanisms of weight gain and of serum cholesterol metabolism are apparently different for the two selected lines used in this study. The observation that leaner individuals seem less likely, in general, to have elevated SCL has been made numerous times in the literature (Kritchevsky, 1958), but the explicit understanding of the intake, biosynthesis and metabolism of cholesterol is not yet complete.

Phase Two - Evaluation of Genetic Parameters

Phase two of this study included raising and testing a generation of unselected male mice, randomly mating them to contemporary females, and raising and testing the male offspring of these matings in the same manner that the sires had been tested. This experimental design allowed the estimation of heritability by a sire-son regression, calculation of genetic correlations from the data of the sire and son generations and calculation of phenotypic correlations from the pooled data of the sires and sons. The traits measured in this phase of the study were SCL, voluntary physical activity, body weight, weight gain and body moisture content. The means, standard deviations, and coefficients of variation of these traits for the sires, the sons, and the pooled data are listed in Table 11.

Heritability Estimates

Heritability estimates of the traits measured in this phase of the study were obtained by a regression of son records on sire records (Table 12). A total of 194 sires and 466 sons completed the entire test

TABLE 11. MEANS, STANDARD DEVIATIONS AND COEFFICIENTS OF VARIATION FOR TRAITS MEASURED IN THE SIRE POPULATION, THE SON POPULATION AND THE POOLED POPULATION

GENETIC PARAMETERS STUDY

Trait	Sires (194)[1]			Sons (466)			Pooled (660)		
	Mean	S.D.	C.V.	Mean	S.D.	C.V.	Mean	S.D.	C.V.
SCL (mg/100 ml)	138.9	36.0	26.0	115.2	27.7	24.0	122.4	32.5	26.6
Body Moisture (%)	65.1	2.4	3.7	64.0	2.9	4.6	64.3	2.8	4.4
Activity 28 (revolutions)	4440	2641	59.5	4791	3488	72.8	4693	3269	69.6
Activity 49 (revolutions)	9263	4666	50.4	9842	5478	55.7	9677	5264	54.4
Activity 70 (revolutions)	8973	5158	57.5	13848	7560	54.6	12390	7294	58.9
Total Activity (revolutions)	22675	9865	43.5	28482	13427	47.1	26760	12786	47.8
Weight 21 (g)	13.2	1.7	12.9	13.3	1.6	11.8	13.3	1.6	12.1
Weight 44 (g)	31.9	2.8	8.6	31.0	2.6	8.4	31.2	2.7	8.5
Weight 67 (g)	35.0	3.2	9.2	33.9	2.7	8.1	34.2	2.9	8.5
Gain 21-44 (g)	18.7	2.4	12.7	17.6	2.4	13.8	17.9	2.5	13.8
Gain 44-67 (g)	3.1	2.5	82.6	2.9	2.0	67.6	3.0	2.2	72.6
Gain 21-67 (g)	21.8	2.9	13.4	20.6	2.6	12.6	20.9	2.7	13.1

[1](N) = number of observations per mean.

TABLE 12. HERITABILITIES AND STANDARD ERRORS OF TRAITS IN GENETIC PARAMETERS STUDY

Trait	h^2	S.E.
SCL	.31	.07
Body Moisture	.29	.11
Activity 28	.31	.12
Activity 49	.50	.11
Activity 70	.27	.14
Total Activity	.54	.12
Weight 21	.11	.09
Weight 44	.33	.09
Weight 67	.42	.08
Gain 21-44	.24	.09
Gain 44-67	.22	.07
Gain 21-67	.27	.08

and, using method number one of Kempthorne and Tandon (1953), the record of every sire was repeated with each of his sons, resulting in a total of 466 sire-son pairs in the regression.

The heritability estimate of serum cholesterol obtained in this study, .31 $\pm$ .07, was decidedly lower than the estimate of .51 $\pm$ .05 found by Weibust (1973) for male mice. The discrepancy is not unusual, however, as Weibust (1973) reported realized heritability in mice selected for maximum and minimum plasma cholesterol levels while this study estimated heritability of SCL from a sire-son regression on an unselected random-bred line of mice. Also, differences in the lines used, environment and laboratory procedures undoubtedly affected the estimates since heritability estimates are peculiar to the population from which they are obtained. Although few other estimates of heritability of SCL in mice were found in the literature, the figure obtained agreed well with estimates obtained in chickens (Cherms et al., 1960; Wilcox et al., 1963; Estep et al., 1969). The estimates of heritability of SCL in beef cattle (Stufflebean and Lasley, 1969) and squirrel monkeys (Clarkson et al., 1971), .80 and .92, respectively, were much higher than the estimates obtained for mice in this study. The discrepancies were, presumably, attributable to species variation and to differing methods of heritability estimation.

The importance of the results obtained in this study is the agreement with past literature that circulating cholesterol levels are at least moderately heritable. The advantage of such knowledge in relation to humans is that individuals whose close relatives are known to suffer from elevated levels of circulating cholesterol can be observed closely

and treated quickly for symptoms of this condition. By following such procedures, the prevention of severe cases of atherosclerosis may become more successful.

Although no estimates of the heritability of voluntary physical activity were found in the literature, reports indicate that activity is subject to a moderate degree of genetic control. Thompson (1956) found significant strain differences in relative exploratory tendency of mice, indicating that inheritance definitely influenced this behavioral trait. From a study involving two inbred strains of mice, F_1 progeny, F_2 progeny and backcrosses with parental strains, McClearn (1961) concluded that the mode of inheritance of activity was probably dominance. Bruell (1964) reported "behavior hybrid vigor" in spontaneous wheel-running. He found that a significant number of mice obtained activity scores above that of their more active parent, and interpreted the results as evidence of heterosis in the trait.

In this study the heritabilities of voluntary physical activity were estimated in mice from data collected on 28, 49 and 70 days of age and from a total activity score (the sum of the three scores of each individual). The values of heritability obtained ranged from $.27 \pm .14$ to $.50 \pm .11$ for activity scores at various ages and was $.54 \pm .12$ for the total activity score. The range of these estimates certainly concurs with the studies mentioned above in concluding that voluntary physical activity is of moderate to high heritability.

The heritability estimates of postweaning gain are in good agreement with those of Rahnefeld et al. (1963), Vinson et al. (1969), LaSalle et al. (1974), and Jara-Almonte and White (1973). Estimates of body

weight heritability at approximately six weeks of age are also similar to those of Vinson et al. (1969) and Jara-Almonte and White (1973) although estimates at 21 days of age in the present study were somewhat lower than previous figures obtained.

The heritability of body moisture content at maturity, .29 $\pm$ .11, was of similar magnitude to the estimates of body weight and weight gain heritabilities. Thus, in addition to gaining weight at approximately the same rate as their parents, offspring apparently developed in proportionate body composition similarly to their parents. Such a phenomenon, of course, has been demonstrated by the successful selection of mice for large and small weight gains as were used in phase one of this study and by the development of various obese and lean lines of mice.

Phenotypic Correlations

The data from the genetic phase of this study were used to obtain some general phenotypic correlations among the traits observed. The information from 194 sires and 466 sons from the unselected, random-bred population was pooled for a total of 660 observations on each trait. Product-moment correlations were calculated among the following traits: SCL, body moisture content, activity scores at 28, 49, and 70 days of age, a total activity score (the sum of the three individual scores), body weight at 21, 44, and 67 days of age,and changes in body weight from 21 to 44, 44 to 67, 21 to 67 days of age. The resulting correlations are listed in Table 13 and are discussed below.

Each activity score was highly, positively correlated with every other activity score. There were no significant correlations between

TABLE 13. PHENOTYPIC CORRELATIONS -- GENETIC PARAMETERS STUDY

	SCL	Body Moisture	Activity 28	Activity 49	Activity 70	Total Activity	Weight 21	Weight 44	Weight 67	Gain 21-44	Gain 44-67	Gain 21-67
SCL	1	-.02	-.01	.04	-.04	-.01	.03	.17**	.05	.16**	-.14**	.03
Body Moisture		1	-.05	.00	-.04	-.03	-.15**	-.09*	-.11*	.00	-.04	-.03
Activity 28			1	.43**	.33**	.62**	.12**	.07	.03	-.00	-.05	-.04
Activity 49				1	.54**	.83**	.09*	-.03	-.08	-.10*	-.07	-.14**
Activity 70					1	.88**	.10*	-.02	-.10*	-.08	-.12**	-.17**
Total Activity						1	.13**	-.00	-.08	-.09	-.11*	-.16**
Weight 21							1	.42**	.38**	-.20**	-.01	-.18**
Weight 44								1	.70**	.81**	-.28**	.50**
Weight 67									1	.51**	.48**	.84**
Gain 21-44										1	-.30**	.66**
Gain 44-67											1	.52**
Gain 21-67												1

*(P < .05) **(P < .01)

activity and SCL. This agreed with the data in the characterization of line differences (first phase of the study) where SCL and activity were correlated significantly in the high and low selected lines but not in the unselected control. No significant relationship was found between activity and body moisture content.

Negative correlations were found between activity scores and gains in body weight. In general, these correlations were not significant at the younger ages (i.e., in activity scores at 28 days of age and in weight gains from 21 to 44 days of age), but became highly significant as the mice matured. This suggested that relatively greater total increases in weight are associated with lower activity scores and, conversely, that smaller total weight gains are associated with a greater tendency to exercise. These results are analogous to the conclusions reached in the characterization of line differences (phase one) where animals selected for maximum rate of gain were comparatively lethargic and animals selected for minimum rate of gain were much more active. In the genetic phase of this study, rather than observing differences among lines, the variation within one unselected line provided concurrent results.

An interesting trend occurred in comparing activity scores with body weight at certain ages. The body weight at 21 days of age was significantly, positively correlated (at the $P < .05$ level or higher) with all activity scores. Weight at 44 and 67 days of age were positively (but not significantly) correlated with activity at 28 days of age. However, weights at 44 and 67 days of age were negatively correlated with the

remaining activity scores (at 49 and 70 days of age and with total activity). This information agrees with results obtained by Thye (1973) indicating that mice must reach a certain, minimum weight by their first activity period in order to be successful in turning the exercise wheel effectively. In working with the same selected lines as those employed in the first phase of this study, Thye found that the initial activity scores of the low line mice were below those of the high line mice, presumably because the low line mice did not have the weight and/or stamina necessary to turn the exercise wheels. After this initial period, however, the low line mice obtained much higher activity scores than did the high line mice (which agreed with the results of the first phase of this study). The significant positive correlation between weight at 21 days of age and all activity scores in this study suggests that the mice which weighed comparatively more at 21 days of age were more active than those of lesser body weight. However, the negative trend in the correlations of weights at 44 and 67 days of age indicated that heavier weight at these ages were associated with lower activity scores (i.e., heavier individuals after 44 days of age were less inclined to exercise).

The relationships among the weight traits indicated that mice could be separated into two groups according to the period of life during which they gained weight most rapidly. It appeared that rapid growth in these animals occurred either immediately postweaning (from 21 to 44 days of age) or just after sexual maturity (from 44 to 67 days of age). Two supporting arguments for this conclusion will be presented. First, there were highly significant, positive correlations among body weights

at 21, 44 and 67 days of age. The correlations of total weight gain (from 21 to 67 days of age) with gains immediately after weaning (21-44 days) and with gains after sexual maturity (44-67 days) were significant and positive. However, the correlation between immediate postweaning gain and post-sexual maturity gain was a highly significant, negative value. Thus, the mice gained rapidly either from 21 to 44 or from 44 to 67 days of age. A second piece of evidence exists which indicates that mice gain weight rapidly during either 21-44 or 44-67 days of age. Body weight at 44 days of age was significantly, positively correlated with change in weight immediately after weaning (21 to 44 days of age) but was highly, negatively correlated with change in weight after sexual maturity (44 to 67 days of age). The mice with a high body weight at 44 days of age, apparently, gained that weight immediately postweaning and did not gain much more weight from 44 to 67 days of age. Again, the results obtained in the unselected line of this phase are analogous to the high and low lines in the first phase of the study. There, the high line mice gained rapidly immediately after weaning (as they were selected to do) while the low line mice, which were selected for minimum rate of gain immediately after weaning, were found to gain weight more rapidly after approximately 50 days of age.

SCL, as mentioned above, was not significantly correlated with any of the activity scores in the unselected population. No significant relationship between body moisture content and SCL occurred. The association of serum cholesterol and the weight traits are quite interesting, however. In general, positive correlations were found

between SCL and body weight but the only significant correlation occurred between SCL and weight at 44 days of age ($P < .01$). A highly significant positive correlation was found between SCL and weight gain from 21 to 44 days of age (immediately after weaning) and a highly significant negative correlation occurred between SCL and weight from 44 to 67 days of age (just after sexual maturity). This indicates that mice which gained weight rapidly in the first three weeks after weaning had higher SCL at 62 days of age than mice which gained weight after sexual maturity (after six weeks of age). Again, an analogy can be drawn between this phase of the study and the phase using selected lines. There, the high line mice generally had elevated SCL as compared to the low line mice. The low line mice experienced an increase in weight after approximately 50 days of age in phase one and, in general, had a lower SCL.

No significant relationships were found between body moisture content and SCL, the activity scores, or changes in body weight. Significant negative correlations occurred, however, between moisture content and body weight. The percentage of moisture is inversely related to the amount of fat in total body composition (Lang and Legates, 1969; White, 1975). In general, mice with greater percentage of body moisture had lower percentage of body fat and weighed less than mice with lower percentage of body moisture (and greater body fat percentage). Thus, mice which weighed more generally had deposited a greater percentage of body fat than individuals of lighter body mass.

Genetic Correlations

Genetic correlations and standard errors (Table 14) were calculated from the data obtained in phase two of this study by the methods of Becker (1967).

TABLE 14. GENETIC CORRELATIONS AND STANDARD ERRORS -- GENETIC PARAMETERS STUDY

	SCL	Body Moisture	Activity 28	Activity 49	Activity 70	Total Activity	Weight 21	Weight 44	Weight 67	Gain 21-44	Gain 44-67	Gain 21-67
SCL	1	-.70±.11	-.15±.21	.18±.15	.20±.23	.13±.16	.64±.18	.23±.16	.09±.14	.01±.21	-.15±.19	-.11±.18
Body Moisture		1	-.17±.27	-.07±.20	.78±.12	.22±.20	-.00±.40	-.32±.20	-.56±.13	-.44±.23	-.54±.18	-.75±.10
Activity 28			1	.96±.01	.94±.04	.99±.00	.37±.35	.17±.22	-.07±.19	.06±.28	-.35±.23	-.22±.23
Activity 49				1	.87±.06	.97±.01	.09±.30	-.16±.16	-.16±.14	-.26±.19	-.06±.19	-.24±.17
Activity 70					1	.96±.02	-.16±.44	.02±.26	-.11±.21	.10±.31	-.22±.28	-.10±.27
Total Activity						1	.06±.31	-.03±.17	-.13±.14	-.07±.21	-.18±.19	-.19±.18
Weight 21							1	.84±.10	1.00±.00	.68±.22	.64±.22	1.00±.00
Weight 44								1	.82±.05	.97±.01	.11±.21	.82±.06
Weight 67									1	.65±.11	.66±.10	1.00±.00
Gain 21-44										1	-.14±.25	.64±.14
Gain 44-67											1	.66±.12
Gain 21-67												1

SCL and body moisture were highly, negatively correlated (-.70 $\pm$ .11). The inverse relationship between body moisture and body fat percentage (Lang and Legates, 1969; White, 1974) led to the conclusion that SCL and percentage body fat are positively correlated genetically.
Similar results were obtained in phase one where mice in the high line (i.e., mice with relatively large percentages of body fat) experienced elevated SCL as compared to the low line mice. It is interesting to note that the genetic correlation between SCL and activity in this random bred population are quite small and that SCL is moderately correlated genetically with body weight immediately after weaning and highly correlated with body fat percentage. These results suggest that high weaning weight and high body fat percentage rather than low levels of voluntary physical activity were genetically associated with elevated SCL.

Each activity score was highly correlated genetically with every other activity score. These genetic correlations ranged from .87 $\pm$ .06 to .99 $\pm$ .00. The existence of genetic control of voluntary physical activity was reported in previous literature (Thompson, 1956; McClearn, 1961; Bruell, 1964). The significant line differences in phase one and the heritability, phenotypic and genetic correlations in phase two of this study further substantiated that such genetic control of activity does exist.

Although the genetic correlations between activity and the weight traits were generally negative, they were small in magnitude with rather large standard errors, so that specific conclusions could not be drawn.

A large positive correlation occurred between body moisture and activity at 70 days of age, indicating that sires with high body moisture

percentage (and low fat percentage) produced sons which exercised more than sons whose sires had comparatively greater percentage of body fat. These results concur with the findings of phase one of this study where the low line (low body fat percentage) mice were more active than the high line mice. Body moisture was negatively correlated with weight at 67 days of age and with all the weight gains.

Large genetic correlations were found between body weights at 21, 44 and 67 days of age. Changes in body weight were positively correlated except for the correlation between gain from 21 to 44 days of age and gain from 44 to 67 days of age (which was a small, negative correlation). The correlations between weights and changes in body weight were all high, positive correlations (ranging from .64 $\pm$ .22 to 1.00 $\pm$ .00) except for the correlation between weight at 44 days of age and gain from 44 to 67 days of age (a small, positive correlation). Although caution must be used in interpreting correlations such as these two (weight at 44 with gain from 44 to 67 days of age and gain from 21 to 44 with gain from 44 to 67 days of age) because of their large standard errors, the trend is exactly the same as that found in the phenotypic correlations. The interpretation was that the mice appeared to gain weight either immediately postweaning (21 to 44 days of age) or just after sexual maturity (44 to 67 days of age).

GENERAL DISCUSSION

The degree to which circulating cholesterol levels are governed by various genetical and physiological factors is a concept of considerable importance in the understanding of atherosclerosis. Elevated levels of cholesterol in the circulatory system have been associated with increased incidence of atherosclerotic plaques in the blood and lymph vessels in several species, including humans (Kritchevsky, 1958); squirrel monkeys (Clarkson et al., 1971); and pigeons (Wagner and Clarkson, 1974). As a result of this association, a great deal of effort has been expended in observing the sources of exogenous and endogenous cholesterol, metabolism of cholesterol in the body and physiological factors which are linked with the relative increase or decrease in the levels of circulating cholesterol (e.g., heredity, diet, activity, and stress). Only when the complex balance of cholesterol in the various tissues, organs and systems of the body is thoroughly understood can acceptable progress in the treatment and prevention of atherosclerosis be realized.

The purpose of this study was to record the level of serum cholesterol associated with varying degrees of activity, total intake, diet, weight gain and body moisture content. Due to the relative ease of maintenance, reduced costs and shortness of generation interval, laboratory mice were a suitable species with which to conduct this study.

Serum cholesterol levels were found to be decisively influenced by heredity in two separate phases of the study. While working with two

lines of mice selected for maximum and minimum rate of weight gain and an unselected control, highly significant line differences indicated the existence of genetic control of the trait. A sire-son regression in the random-bred control population yielded a heritability estimate of $.31 \pm .07$, confirming the thesis that SCL is moderately heritable.

The levels of SCL in the two selected lines of mice were quite divergent. Those animals which had been selected for 22 generations for maximum rate of gain from 21 to 42 days of age were found to have significantly higher SCL than the mice selected for minimum rate of gain in all except one instance. The positive association between SCL and gain in body weight from 21 to 44 days of age was also indicated by the phenotypic correlations of the random-bred line used in phase two of the study.

The extent to which SCL was affected by various levels of intake and by amounts of fat in the diet was considerable. Greater intake was associated with elevated SCL regardless of the type of diet consumed. Animals consuming an 11% fat diet experienced much greater SCL than animals fed a 4-1/2% fat diet across all lines. Since both of these diets were well within a normal range of composition for the mice, the effect of feeding an abnormally high fat percentage diet or of feeding dietary cholesterol by other investigators would undoubtedly produce a tremendous elevation in SCL (Lewis et al., 1961; Hanson et al., 1967; Reiser, 1973; Clarkson et al., 1971; Quintano et al., 1971; Wagner et al., 1973; Wagner and Clarkson, 1974). Another consideration in the effects of diet on SCL concerns the amount of protein consumed. The difference between the fat percentages of the two diets was made up in protein.

Several workers have reported that decreased protein in the diet causes an increase in SCL (Leveille and Sauberlich, 1964; Nishida et al., 1969; Coccodrilli et al., 1970). Thus, the increased SCL of the mice consuming 11% fat diet may have been due to increased fat percentage, decreased protein percentage or to a combination of the two factors. Regardless of which factor caused the change, SCL was definitely influenced by diet.

An association was found between SCL and voluntary physical activity. Animals which were comparatively active consistently had much lower SCL than animals which were relatively lethargic. From the design of this study it could not be determined whether reduced SCL led to greater activity or whether a high level of activity caused reduced SCL. The negative correlation between the two traits was highly significant, however, and substantiates previous studies in which high levels of exercise caused a stablization or reduction in circulating cholesterol levels (Papadopoulous et al., 1969; Lewis et al., 1961; Jones et al., 1964).

The results of this study suggest several factors which may be used in the control of SCL. The genetic manipulation of controlling SCL is a reasonable concept in animals since the trait is moderately heritable. Such manipulation would be useful in the rearing of pigeons, squirrel monkeys and other species which are prone to severe atherosclerosis and is certainly of importance in the use of laboratory animals for further research on SCL. For example, development of strains of animals selected for high and low SCL should be quite effective and various data collected on such selected strains can contribute considerably to the understanding of SCL. Although genetic manipulation for reduced SCL is

not feasible in humans, the knowledge that elevated SCL is heritable will allow rapid treatment of individuals prone to atherosclerosis.

Other factors indicated in this study to be useful in the control of SCL are composition of diet, level of intake and amount of activity. If the exact causes for the influence of these factors on SCL can be documented, relatively simple forms of treatment may be greatly successful in the treatment and prevention of elevated SCL. Simple treatment here refers to such concepts as dietary regime and systematic exercise programs which would not normally require any medication and could be successful with a moderate amount of professional supervision.

SUMMARY

Feed intake, growth, efficiency and serum cholesterol levels (SCL) were recorded for 102 male mice and voluntary physical activity and SCL were recorded for 113 male mice in phase one of this study. These animals were taken from lines selected for 22 generations for maximum 21- to 42-day gain (H), minimum 21- to 42-day gain (L), and an unselected control (C). Heritability (h^2), phenotypic and genetic correlations among SCL, activity, growth and body moisture content were estimated for 466 sire-son pairs of mice from a random-bred control line in phase two.

In the line characterization study, phase one, a least squares analysis of variance from a model including lines, rations and line x ration interactions indicated highly significant differences in the main effects and few significant interactions. Total feed intake for 7 weeks in animals fed a high fat diet (11% fat) for line H, C, and L was 316.5 g, 264.8 g and 212.0 g; total digestible nutrient intake was 278.5 g, 233.0 g, and 186.6 g; total weight gain was 37.0 g, 20.8 g and 12.9 g; feed efficiency was 11.7%, 7.9%, and 6.1%. SCL (mg/100 ml) at 70 days of age for these animals was 220.3, 178.1, and 180.4. Animals fed a control diet (4.5% fat) had total feed intake for 7 weeks of 363.2 g, 293.2 g, and 238.0 g; total digestible nutrient intake was 272.4 g, 219.9 g, and 178.5 g; total weight gain was 33.7 g, 19.9 g, and 11.6 g; total feed efficiency was 9.3%, 6.8%, and 4.9%. SCL for these animals at 70 days of age was 143.3, 124.1, and 132.2. The total activity scores (revolutions) for the H, C and L lines were 79234, 107987 and 124409 and their 70-day SCL's (mg/100 ml) were 155.0, 130.5 and 117.6. Activity

generally increased in all lines from 21 to 56 days of age, then declined slightly. During each period line L showed the greatest tendency to exercise, line H the least and line C an intermediate amount. Negative correlations occurred between SCL and activity in the high and low lines. SCL was negatively correlated with intake but positively correlated with efficiency in the high line. Total gain was positively correlated with total intake and with total efficiency in all lines.

In phase two, estimates of h^2 were obtained by a sire-son regression in which the record of the sire was repeated for each of his sons. Heritability estimates of SCL and body moisture content were .31 $\pm$ .07 and .29 $\pm$ .11, and heritability estimates of activity ranged from .27 $\pm$ .14 to .54 $\pm$ .12. The moderate to high estimates obtain in this study indicate that SCL and activity are considerably influenced by genetic control. Heritability estimates of weight gain ranged from .22 $\pm$ .07 to .27 $\pm$ .08 and heritability estimates of body weight ranged from .11 $\pm$ .09 to .42 $\pm$ .08.

Phenotypic correlations between body moisture content and body weights were negative and significant, indicating that individuals of heavier body weight had lower moisture percentage and, consequently, higher fat percentage (due to the inverse relationship between body fat and body moisture). Phenotypic correlations between activity and gains in body weight were generally negative and were significant after approximately 44 days of age. Although activity and SCL were not genetically correlated, large negative genetic correlations occurred between body moisture content, SCL and body weight. These results indicated that high levels of body fat and weight are probably associated

with elevated SCL to a greater extent genetically than are low activity levels.

REFERENCES

Becker, W. A. 1967. Manual of procedures in quantitative genetics. Washington State University Press, Pullman, Washington.

Bohren, B. B., H. E. McKean and Y. Yamada. 1961. Relative efficiencies of heritability estimates based on regression of offspring on parent. Biometrics 16:481-491.

Brainard, J. B. 1959. Effect of prolonged exercise on atherogenesis in the rabbit. P.S.E.B.M. 100:244-246.

Bruell, J. H., A. F. Daroczy and H. K. Hellerstein. 1962. Strain and sex differences in serum cholesterol levels of mice. Science 135:1071-1072.

Bruell, J. H. 1963. Additive inheritance of serum cholesterol level in mice. Science 142:1664-1665.

Bruell, J. H., 1964. Heterotic inheritance of wheelrunning in mice. J. Comp. and Physiol. Psych. 58(1):159-163.

Cherms, F. L., F. H. Wilcox and C. S. Shaffner. 1960. Genetic studies of serum cholesterol level in the chicken. Poul. Sci. 39:889-892.

Clarkson, T. B., H. B. Lofland, Jr., B. C. Bullock and H. O. Goodman. 1971. Genetic control of plasma cholesterol. Archives of Path. 92:37-45.

Coccodrilli, G. D., Jr., P. T. Chandler, and C. E. Polan. 1970. Effects of dietary protein on blood lipids of the calf with special reference to cholesterol. J. Dairy Sci. 53(11):1-5.

Driscoll, J. L., D. Aubuchon, M. Descoteaux and H. F. Martin. 1971. Semiautomated, specific routine serum cholesterol determination by gas-liquid chromatography. Analytical Chem. 43(10):1196-1200.

Dupont, J. 1965. Relationship between utilization of fat and synthesis of cholesterol and total lipid in young female rats. J. Am. Oil Chemists Soc. 42:903-907.

Eapen, K. J., O. B. Goswami and S. K. Pillai. 1971. Inheritance of serum cholesterol and its relation to body weight in white mice. J. Genet. 60(3):222-229.

Estep, G. D., R. C. Fanguy and T. M. Ferguson. 1969. The effect of age and heredity upon serum cholesterol levels in chickens. Poul. Sci. 58:1908-1911.

Falconer, D. S. 1953. Selection for large and small size in mice. J. Genetics 51:470-501.

Falconer, D. S. 1960. Introduction to Quantitative Genetics. Ronald Press, New York.

Hanson, D. L., J. A. Lorenzen, A. E. Morris, R. A. Ahrens and J. E. Wilson, Jr. 1967. Effects of fat intake and exercise on serum cholesterol and body composition of rats. Am. J. Physiol. 213(2):347-352.

Hazel, L. N. 1943. The genetic basis for constructing selection indexes. Genetics 28:476-490.

Jara-Almonte, M. and J. M. White. 1973. Genetic relationships among milk yield, growth, feed intake, and efficiency in laboratory mice. J. An. Sci. 37(2):410-416.

Jones, E. M., H. J. Montoye, P. B. Johnson, Sr. M. J. M. Martin, W. D. Van Huss and D. Cederquist. 1964. Effects of exercise and food restriction on serum cholesterol and liver lipids. Am. J. Physiol. 207(2):460-466.

Kempthorne, O. and O. B. Tandon. 1953. The estimation of heritability by regression of offspring on parent. Biometrics 9:90-100.

Kritchevsky, D. 1958. Cholesterol. Wiley, New York.

Kohn, H. I. 1950. Changes in plasma of the rat during fasting and influence of genetic factors upon sugar and cholesterol levels. Am. J. Physiol. 163:410-417.

Lang, B. J. and J. E. Legates. 1969. Rate, composition and efficiency of growth in mice selected for large and small body weight. Theoretical and Applied Genetics 39:306-314.

LaSalle, T. J., J. M. White and W. E. Vinson. 1974. Direct and correlated responses to selection for increased postweaning gain in mice. Theoretical and Applied Gen. 44:272-277.

Leveille, G. A. and H. E. Sauberlich. 1964. Plasma and liver lipids of mice as influenced by dietary protein and sulfur-containing amino acids. J. Nutr. 84:10-14.

Levin, L. 1945. The effects of several varieties of stress on the cholesterol content of the adrenal glands and of the serum of rats. Endocrinology 37:34-43.

Lewis, L. A., I. H. Page and H. B. Brown. 1961. Effect of exercise on serum and hepatic lipids in rats fed high fat diets. Am. J. Physiol. 201(1):4-8.

Maynard, L. A. and J. K. Loosli. 1969. Animal Nutrition. McGraw Hill Book Company, 6th ed.

McClearn, G. E. 1961. Genotype and mouse activity. J. Comp. and Physiol. Psychol. 54(6):674-676.

Nishida, T., A. Ueno and F. A. Kummerow. 1960. Effect of dietary protein on the metabolism of sodium acetate-1-C^{14} in chicks. J. Nutr. 74:379-386.

Papadopoulos, N. M., C. M. Bloor and J. C. Standefer. 1969. Effects of exercise and training on plasma lipids and lipoproteins in the rat. J. Appl. Physiol. 26(6):760-763.

Quintao, E., S. M. Grundy and E. H. Ahrens, Jr. 1971. Effects of dietary cholesterol on the regulation of total body cholesterol in man. J. Lipid Res. 12:233-247.

Rahnefeld, G. W., W. J. Boylan, R. G. Comstock and Madho Singh. 1963. Mass selection for postweaning growth in mice. Genetics 48:1567-1583.

Reeve, E. C. R. 1955. The variance of the genetic correlation coefficient. Biometrics 11:357-374.

Reiser, R. 1973. Saturated fat in the diet and serum cholesterol concentration: a critical examination of the literature. Am. J. Clin. Nutr. 26(5):524-555.

Riley, V. 1960. Adaptation of orbital bleeding technic to rapid serial blood studies. P.S.E.B.M. 104:751-754.

Robertson, A. 1959. The sampling variance of the genetic correlation coefficient. Biometrics 15:469-485.

Stufflebean, C. E. and J. F. Lasley. 1969. Hereditary basis of serum cholesterol level in beef cattle. J. Hered. 60:15-16.

Sure, B., M. C. Kik and A. E. Church. 1933. The influence of fasting on the concentration of blood lipids in the albino rat. J. Biol. Chem. 103:417-424.

Thompson, W. R. 1956. The inheritance of behavior. Activity differences in five inbred mouse strains. J. Hered. 47:147-148.

Thye, F. W. 1973. Personal communication.

Vinson, W. E., E. J. Eisen and O. W. Robison. 1969. Predicted response to selection for crossbred performance in mice. J. An. Sci. 28(6):725-733.

Wagner, W. D. and T. B. Clarkson. 1974. Mechanisms of the genetic control of plasma cholesterol in selected lines of show racer pigeons. P.S.E.B.M. 145:1050-1057.

Wagner, W. D., T. B. Clarkson, M. A. Feldner and R. W. Prichard. 1973. The development of pigeon strains with selected atherosclerosis characteristics. Exp. Mol. Pathol. 19:304-319.

Weibust, R. S. 1973. Inheritance of plasma cholesterol levels in mice. Genet. 78:303-312.

White, J. M. 1974. Body composition in mice selected for growth. An. Sci. 39:152. (Abstr.)

Wilcox, F. H., F. L. Cherms, Jr., L. D. Van Vleck, W. R. Harvey and C. S. Shaffner. 1963. Estimates of genetic parameters of serum cholesterol level. Poul. Sci. 42:37-42.

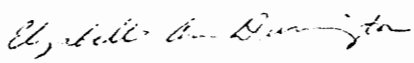
Yamamoto, R. S., L. B. Crittenden, L. Sokoloff and G. E. Jay, Jr. 1963. Genetic variations in plasma lipid content in mice. J. Lipid Research 4(4):413-418.

VITA

Elizabeth Ann Dunnington, daughter of Francis Alden and Ann Terrell Dunnington, was born in Buffalo, New York on August 12, 1950. She completed her secondary education at Franklin High School, Franklin, New Jersey, after attending schools in New York, North Carolina and New Jersey.

The author attended Virginia Polytechnic Institute and State University, Blacksburg, Virginia, from September 1968 until July 1973, obtaining a Bachelor of Science degree with distinction from the Animal Science Department. Returning to Virginia Polytechnic Institute and State University in September 1973, the author began working towards a Master of Science degree in Dairy Science (genetics).

The author is a member of Phi Kappa Phi and Phi Sigma honor societies.



LINE CHARACTERIZATION AND EVALUATION OF GENETIC PARAMETERS OF SERUM CHOLESTEROL LEVELS, ACTIVITY, FEED INTAKE, GROWTH AND BODY MOISTURE IN SELECTED AND UNSELECTED LINES OF LABORATORY MICE

by

Elizabeth Ann Dunnington

(ABSTRACT)

Feed intake, growth, efficiency and serum cholesterol levels (SCL) were recorded for 102 male mice, and voluntary physical activity and SCL were recorded for 113 male mice from lines selected for 22 generations for maximum 21- to 42-day gain (H), minimum 21- to 42-day gain (L), and a random-bred control (C). Heritability (h^2), phenotypic and genetic correlations of SCL, activity, growth and body moisture content were estimated for 466 sire-son pairs of mice from an unselected line. Blood samples were obtained by sinus orbital bleeding at 35 and 70 days of age in the line characterization study and at 62 days of age in the genetic parameters study and were analyzed for SCL by gas chromatography. Voluntary physical activity was recorded as revolutions of an exercise wheel every sixth day from 21 to 70 days of age in the line characterization study and at 28, 49, and 70 days of age in the genetic parameters study. Feed intake (of an 11% fat, 9% fat, and 4-1/2% fat diet) was recorded for several days each week and extrapolated to obtain weekly intake data. A least squares analysis of variance from a model including lines, diets, and line x diet interactions indicated highly significant differences in the main effects but few significant interactions. Total SCL, growth and efficiency were higher and intake lower in mice on the higher fat diet. SCL (mg/100 ml) at 10 weeks of age was

220.3, 178.1, and 180.4 for animals fed an 11% fat diet and 143.3, 124.1, and 132.2 for animals fed a 4.5% fat diet. Activity scores (revolutions) for the H, C, and L lines were 79234, 107987, and 124409. Activity generally increased in all lines from 21 to 56 days of age, then declined slightly. Heritability estimates of SCL and body moisture content were .31 $\pm$ .07 and .20 $\pm$ .11, and h^2 estimates for activity ranged from .27 $\pm$ .14 to .54 $\pm$ .12. Negative genetic correlations occurred between body moisture content and SCL and between body moisture content and weight gains.